Henzel, Andréia; Sperotto Brum, Mário Celso; Lovato, Luciane Teresinha; Weiblen, Rudi
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Universidade Federal do Rio Grande do Sul
Porto Alegre, Brasil

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Serological Survey of Feline Calicivirus and Felid Herpesvirus in Rio Grande do Sul, Brazil

Andréia Henzel1,4, Mário Celso Sperotto Brum2, Luciane Teresinha Lovato1,4 & Rudi Weiblen4

ABSTRACT

Background: Feline calicivirus (FCV) and felid herpesvirus type 1 (FeHV-1) are widely distributed in the feline population. These viruses are the main cause of upper respiratory tract disease in this species, and FCV can also cause oral disease characterized by stomatitis and ulcers. Furthermore, FCV has been associated with a systemic hemorrhagic syndrome named FCV-associated virulent systemic disease (FCV-VSD), which has not yet been reported in Brazil. The aim of the present study was to investigate the presence of antibodies against FCV and FeHV-1 in the population of domestic felines from some counties of the Rio Grande do Sul State (RS), using a virus neutralizing (VN) assay.

Materials, Methods & Results: A total of 630 feline serum samples collected between the years 2007 and 2011 were analyzed to detect antibodies against FCV and FeHV-1 by a VN assay. The serum samples came from cats admitted in veterinary clinics, household cats and cats examined in veterinary hospitals at three universities from Rio Grande do Sul State (RS) [UFRGS, UFSM and UPF]. The serum samples were classified according to the origin, gender, age and vaccination status of the cat. All animal handling procedures were performed under veterinary supervision and following the recommendations of the Brazilian Committee on Animal Experimentation. The feline cell line CRFK (Crandell-Rees feline kidney) was used for viral amplification and for the VN assay. The viruses used in the assay were the isolate SV65/90 of FCV, and the isolate SV534/00 of FeHV-1, from the Setor de Virologia/ UFSM; which were well characterized in a previous study by our group. The serum samples were tested against 100-200 TCID 50/mL (tissue cellular infection dose 50/mL) of both viruses in the VN assay, and a serial dilution of the serum was performed, starting at 1:5 up to > 1:1280 for FCV and at 1:2 to > 1:256 for FeHV-1 in 96 wells plates. Neutralization titers were calculated as the reciprocal of the highest serum dilution able to inhibit the cytopathic effect. Concerning to the results obtained, the groups with the highest number of collected samples were the group of the male cats (44%, 277/630), the group of the cats with ages among one to five years old (36.3%, 229/630) and the group of non-vaccinated cats (94%, 592/630). Neutralizing antibodies against one or both viruses were detected in 53.6% (338/630) of the 630 cats sampled; 23% (145/630) of the cats were seropositive only to FCV, 14.4% (91/630) were seropositive only to FeHV-1 and 16.2% (102/630) were seropositive for both, FCV and FeHV-1. Regarding the groups, a higher percentage of positive samples was found for the group of female cats (85.6%) and the group of cats over five years old for both viruses (82.3%). Considering vaccination status, only 6% of the cats were vaccinated against FCV and/or FeHV-1, however not all vaccinated cats had neutralizing antibodies against the viruses, since only 50% of the vaccinated population were seropositive against FCV and 42.1% of them were seropositive for FeHV-1.

Discussion: The data showed in the present study demonstrated that both viruses are circulating in the feline population sampled; and, it also revealed that FCV seems to be more prevalent among this population since the presence of antibodies against FCV were detected more frequently than antibodies against FeHV-1. The high number of non-reagent serum and non-vaccinated cats indicates that there is yet a large percentage of the cat population that is susceptible to infection. The application of vaccination programs according of the recommendations could help to prevent the infection and change the situation among the feline population sampled.

Keywords: FCV, FeHV-1, domestic feline, neutralizing antibodies.
INTRODUCTION

Feline calicivirus (FCV) and felid herpesvirus type 1 (FeHV-1) are the main viral agents of upper respiratory tract disease in felines [7,20]. FCV is a non-enveloped RNA virus, member of the Caliciviridae family, and Vesivirus genus [20]. Seven days post-infection with FCV, neutralizing antibodies can be readily detected [15]. The levels of such antibodies correlate positively with protection against a homologous challenge but not with heterologous strains [18]. FeHV-1 is an enveloped DNA virus classified in the Herpesviridae family, Varicellovirus genus [7]. However, unlike FCV, FeHV-1 isolates are more homogeneous; thus, the immunity induced by natural infection or vaccination provides good protection even against heterologous challenge, although latency and viral shedding after reactivation still occur [7].

The presence of infection by both viruses is described worldwide by virus isolation [1,10] and serologic surveys in domestic [11] and wild felines [2,13,17]. The circulation of the viruses in populations of domestic and wild felines in Brazil has already been described through some serologic surveys against FCV and/or FeHV-1 [6,14,22]. The first isolation of FCV was described in the Southern region of Brazil [27] and an epidemiologic survey by our group reported the first isolation of FeHV-1 [12].

The goal of this study was to demonstrate the circulation of FCV and FeHV-1 among the cat population of the central and northern regions of the Rio Grande do Sul State, Brazil, using the neutralizing antibody assay. An extensive and comprehensive survey for all the state or even the region presented in this article had not been performed previously.

MATERIALS AND METHODS

Serum samples

Serum samples from domestic cats were examined for the presence of neutralizing antibodies to feline calicivirus (FCV) and felid herpesvirus type 1 (FeHV-1) using a virus neutralization (VN) assay. The serum samples were collected between the years 2007 and 2011 from the following cities: Santa Maria, Passo Fundo, Porto Alegre and surrounding areas. The serum samples were obtained from the veterinary hospitals (VH) of the Universidade Federal de Santa Maria (UFSM), the Universidade Federal do Rio Grande do Sul (UFRGS) and from the Universidade de Passo Fundo (UPF). Samples were also collected from veterinary clinics and household cats from the following cities: Cachoeira do Sul, Cruz Alta, Porto Alegre, Santa Maria and Santo Ângelo. As detailed in Table 1, the groups were classified according to origin, gender, age, and vaccination status. A total of 630 serum samples were examined as described in Table 1. A greater percentage of samples (323/630) came from Santa Maria due to the high number of collections from the Veterinary Hospital of the UFSM; 254 samples came from UPF, 37 samples came from UFRGS, and the remaining 16 samples came from household cats from different cities of the Rio Grande do Sul State (see Table 1).

Cells and viruses

The feline kidney cell line CRFK (Crandell-Rees feline kidney) was used for viral amplification and for the VN assay. Cells were routinely maintained in Eagle’s minimal essential medium (MEM) containing penicillin (1.6 mg/L), streptomycin (0.4 mg/L), amphotericin B (2.0 mg/L) and 10% fetal calf serum. The viruses used in the assay were the FCV isolate SV65/90 and the FeHV-1 isolate SV534/00 from the Setor de Virologia from the UFSM. Both viruses were well characterized in a previous study by Henzel et al. [12].

Virus neutralization assay

The serum samples were tested against 100-200 TCID50/mL of both viruses in the VN assay. The sera were initially inactivated 30 min before use at 56°C. A serial dilution was performed, starting at 1:5 up to > 1:1280 for FCV and starting at 1:2 to > 1:256 for FeHV-1 in a 96 well microplate. The serum and virus mixture was incubated at 37°C for 2 h and then a suspension of CRFK cells was added to each well. The plates were incubated at 37°C and 5% CO2 for three to five days. Neutralization titers were calculated as the reciprocal of the highest serum dilution able to avoid the cytopathic effect.
Table 1. Number of cats serologically positive for neutralizing antibodies against feline calicivirus (FCV) and/or felid herpesvirus type 1 (FeHV-1) according to origin, gender, age, and vaccination status.

<table>
<thead>
<tr>
<th>Origin of serum</th>
<th>Samples (%)</th>
<th>Gender</th>
<th>Age (years) (%)</th>
<th>Vaccination status (%)</th>
<th>Cats serologically positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFSM – VH</td>
<td>323 (51.2)</td>
<td>Female</td>
<td>&lt;1 1.5-5 5-10 &gt;10</td>
<td>Ni No Yes No Yes No</td>
<td>62 14 13 28 7 31 28 9 22 18</td>
</tr>
<tr>
<td>UFRGS – VH</td>
<td>37 (5.8)</td>
<td>Male</td>
<td>12 3-10 &gt;10</td>
<td>Ni No Yes No Yes No</td>
<td>13 4 11 9 20 28 30 10 14 11</td>
</tr>
<tr>
<td>House and veterinary</td>
<td>230 (36.8)</td>
<td>Both</td>
<td>12 3-10 &gt;10</td>
<td>Ni No Yes No Yes No</td>
<td>43 9 21 30 13 39 30 12 15 24</td>
</tr>
<tr>
<td>Total</td>
<td>630</td>
<td></td>
<td></td>
<td></td>
<td>338 145 102 38 145 159 91 102 191 338</td>
</tr>
</tbody>
</table>

RESULTS

The serum samples with positive results to the presence of neutralizing antibodies to feline calicivirus (FCV) and/or felid herpesvirus type 1 (FeHV-1) are described in Table 1 and Figure 1. Also, in the Figure 1 it is described in detail the number of positive samples to each of the viruses according to gender, age and vaccination status. From the 630 samples collected, 338 (53.6%) cats were serologically positive for FCV and/or FeHV-1: 145 (23%) were positive only for FCV, 91 (14.4%) were positive only for FeHV-1 and 102 (16.2%) were positive for both viruses (Table 1).

Regarding the gender of the cats, there were a higher number of samples from male (277) than from female cats (250) [Table 1]. Separating by gender, 85.6% (214/250) of females and 58.8% (163/277) of males (Figure 1) were positive for the presence of antibodies for at least one of the viruses.

The age of the sampled cats varied from one week to 19 years old. To organize the data, the cats were separated into four groups according to age (Table 1 and Figure 1). Most of the cats examined were in the one to five year old group (36.3%) [Table 1], but a higher percentage of positive samples was found for cats over five years old for both viruses. As shown in Figure 1, 82.3% (135/164) of the samples from cats over five years old were positive for FCV and/or FeHV-1 while 70.5% (194/275) of the samples that came from cats under 5 years old were positive.

With regard to the vaccination status, only 6% (38/630) of the tested cat population were vaccinated against FCV and/or FeHV-1 while 94% (592/630) of the samples were from non-vaccinated cats (Table 1). Interestingly, not all vaccinated cats had neutralizing antibodies against the viruses. Of the 38 vaccinated cats, 50% (19/38) were seropositive against FCV and 42.1% (16/38) were seropositive for FeHV-1 (Figure 1).

DISCUSSION

The virus neutralization assay demonstrated that feline calicivirus (FCV) and felid herpesvirus type-1 (FeHV-1) are present in the cat population studied. The frequency of neutralizing antibodies against FCV was higher than that against FeHV-1 (Table 1). This propensity has also been described in other serological surveys performed on domestic felines [14] and wild felines [6,22] in Brazil, as well as in countries in Europe [11,17]. In addition, in surveys including both viruses, a higher rate
of isolation of FCV has been described by researchers from Europe [1,10,25] and Japan [16]. A similar finding was also observed when a virus isolation survey was performed on cats coming from almost the same region as the samples tested in the present study [12].

The apparent difference among the prevalence rates of FCV and FeHV-1 is mostly attributed to the viruses’ biology [10]. The absence of an envelope for FCV may account for its greater resistance in the environment compared to FeHV-1, and this resistance would facilitate the transmission of FCV to susceptible felines [7,20]. Another relevant aspect is the antigenic variability of FCV because it is an RNA virus; in contrast, FeHV-1 has a more stable DNA genome [7,20]. Due to the viral variability of FCV, successive re-infections confer little cross protection to heterologous strains [18,19,23]. Moreover, both viruses are able to induce persistence in the host, although there are basic differences in the way this occurs [8,26]. FeHV-1 is maintained in latency in the neural ganglia, and its recurrence may occur periodically, while FCV is shed continually by the carrier cat [7,8,25]. The constant presence of the virus in the oropharynx, as is the case of FCV, could contribute to a more consistent immune response compared to the periodic contact of the immune system with the virus, as in the case of FeHV-1 [19,21,24].

In addition, there is evidence that the timing of the rise and fall of the immune response is different for these viruses. The rise of serum antibodies is slower for FeHV-1 than for FCV, as demonstrated by the fact that neutralizing antibody titers against FeHV-1 can take more than 40 days to be detected [7,24], while the same detection for FCV takes no more than 7 days after infection or immunization [15]. Furthermore, antibodies against FeHV-1 tend to disappear earlier from the cat serum than antibodies to FCV [7]. Given this difference, cats carrying FeHV-1 but without antibodies against the virus would not be detected by an antibody detection method.

Because only 6% (38/630) of the serum in this study came from vaccinated felines, the presence of neutralizing antibodies against FCV and FeHV-1 in the serum of most of the cats examined indicates natural infection (Table 1). In contrast to what would be expected, not all the samples from vaccinated cats were positive for antibodies. Of the 38 vaccinated cats, 50% (19/38) were seronegative for FCV and 57.9% (22/38) were seronegative for FeHV-1 (Figure 1). The cats included in this survey were considered vaccinated after having received at least one vaccine application in their life, although the details of the vaccination protocols and dates were unknown. The recommended vaccine protocols include applications at 8-9 and 12 weeks of age [20] or at 6, 9 and 12 weeks of age [3], followed by a booster within 12 months after completion of the kitten series and with subsequent revaccinations at intervals of three years or longer [4]. Thus, the absence of antibodies in the serum of these cats could be explained by incorrect vaccination protocols. However, it has to be taken into consideration that the levels of antibodies against FeHV-1 may become undetectable a short time after vaccination (about three months) [8].

The difference in the prevalence of antibodies against either virus among the age groups is demonstrated in Figure 1. Information about the varying prevalence of antibodies among different age groups is rare for the viruses studied in this article. However, there is a general consensus for several other viruses of felines and canines that the prevalence increases with age because older animals have more chance to be exposed to the viruses [5,14]. In our study, there were a slightly higher percentage of FeHV-1 seropositive cats in the group over five years old when compared to cats under this age, 39% and 32%, respectively (data not shown). A high percentage, 50% (27/53), of neutralizing antibodies was also detected against FCV in the group of cats over 10 years old while a very low percentage, 26% (12/46) of cats under one year old were positive (Figure 1). A very interesting finding is that the percentage of antibodies against FeHV-1 is a little higher for cats under one year old compared to FCV, 30.4% and 26%, respectively (Figure 1). These results reinforce data from several isolation surveys where FCV is commonly isolated from adult cats while FeHV-1 is more prevalent among cats under one year old [28].

Neutralizing antibodies against the viruses were detected more often in female cats than male cats, even though the number of samples collected from males was higher (Table 1 and Figure 1). A higher frequency of isolation in females was described for both viruses in a survey conducted by our group in counties of Southern Brazil [12]. Until now, no gender difference has been described in the scientific literature regarding the prevalence of FCV and FeHV-1 as measured by the detection of antibodies or by virus isolation [9,20]. Furthermore, previous articles have pointed out that a high prevalence of the viruses is related to the castration status of the cat, not the gender [1,26].
CONCLUSION

The present study demonstrates the distribution of FCV and FeHV-1 infection among the feline population sampled in part of the central and north regions of the Rio Grande do Sul State. It is also evident that there is a large percentage of the population that is unprotected. Neutralizing antibody detection indicates more extensive contact of this population with FCV than FeHV-1 and a higher prevalence of seropositive female than male cats. The application of vaccination programs according of the recommenda-

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